Lead Toxicity: Current Concerns

by Robert A. Goyer

Over the 20-year period since the first issue of *Environmental Health Perspectives* was published, there has been considerable progress in the understanding of the potential toxicity of exposure to lead. Many of these advances have been reviewed in published symposia, conferences, and review papers in *EHP*. This brief review identifies major advances as well as a number of current concerns that present opportunities for prevention and intervention strategies. The major scientific advance has been the demonstration that blood lead (PbB) levels of 10–15 μg/dL in newborn and very young infants result in cognitive and behavioral deficits. Further support for this observation is being obtained by prospective or longitudinal studies presently in progress. The mechanism(s) for the central nervous system effects of lead is unclear but involve lead interactions within calcium-mediated intracellular messenger systems and neurotransmission. Effects of low-level lead exposure on blood pressure, particularly in adult men, may be related to the effect of lead on calcium-mediated control of vascular smooth muscle contraction and on the renin-angiotensin system. Reproductive effects of lead have long been suspected, but low-level effects have not been well studied. Whether lead is a carcinogen or its association with renal adenocarcinoma is a consequence of cystic nephropathy is uncertain. Major risk factors for lead toxicity in children in the United States include nutrition, particularly deficiencies of essential metals, calcium, iron, and zinc, and housing and socioeconomic status. A goal for the year 2000 is to reduce prevalence of blood lead levels exceeding 15 μg/dL.

Introduction

Over the 20-year period since the first issue of *Environmental Health Perspectives* was published, there has been considerable progress in the understanding of the potential toxicity of exposure to lead. Large numbers of scientists and institutions have contributed to these accomplishments and it is fitting that Volume 100 of *EHP* recognize the achievements that have occurred over the life of the journal. Some 20 or 25 years ago, the pathological descriptions of lead toxicity consisted largely of clinically discernible effects and changes that could be seen in tissues under the microscope (*1*). At that time, permissible occupational exposure to lead was limited to a blood lead (PbB) level of 70–80 μg/dL. PbB levels among people in the general population were 20–30 μg/dL, and levels in children above 40 μg/dL were recognized as indicative of lead toxicity. Nevertheless, over the past 2 decades there has been growing awareness and concern that toxic biochemical and functional effects were occurring at lower levels of exposure than those that produced overt clinical and pathological signs and symptoms. These concerns were expressed in a tentative way in the 1972 National Academy of Sciences/National Research Council report (*2*), which focused on effects of airborne lead in gasoline and in a later World Health Organization Criteria Document (*3*). New terms such as subclinical or asymptomatic lead toxicity were appearing. The National Institute of Environmental Health Sciences (NIEHS) organized one of the first conferences in 1974 to review the emerging clinical and experimental evidence supporting low-level lead health effects, and the proceedings of that conference were published in the seventh issue of *EHP* (*4*). Since that time, *EHP* has published eight other major conferences addressing advances in lead or metal toxicology (*5–13*) and major articles concerning advances in lead research in other issues.

In 1970 the U.S. Surgeon General issued a statement regarding the control of lead poisoning in children that emphasized the need for mass screening of children at risk to excess lead exposure and the need for development of prevention strategies (*14*). In 1975 the Centers for Disease Control in Atlanta recommended that the maximum permissible level of PbB be 30 μg/dL (*15*). This level was revised downward in 1985 to 25 μg/dL (*16*) and again in 1991 (*17*), defining a PbB of 10 μg/dL as an action or intervention level. Perhaps even more importantly, it is now suggested that there may be no level of PbB that does not produce a toxic effect, particularly in the developing central nervous system. This concern applies to the fetus *in utero* and women of childbearing age.

The scientific basis for this recommendation is briefly cited here, but is reviewed in detail in the 1986 Environmental Protection Agency (EPA) Air Quality Criteria for Lead Health Criteria Document (*18*), followed by a supplement in 1989 (*19*). There are also a number of more recent, brief commentaries on health effects of lead in children and adults (*20–24*). Use of the term "low-level lead exposure" refers to levels of lead not associated with overt signs or symptoms of toxicity that might be observed clinically.

The purpose of this brief report is to summarize some of the achievements that have occurred during the life of *EHP* and emphasize topics of present research interest and identify current concerns.

Neuropsychological Effects in Infants and Children

Summary of Studies to Date

The effect of lead on cognitive and behavioral development of the central nervous system (CNS) is the critical effect on infants

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and children and is the focus for current prevention and intervention strategies (*17*). Twenty years ago, the generally recognized effect of lead on the CNS was encephalopathy occurring in children at PbB of 80 μg/dL or greater. This was characterized clinically by ataxia, coma, and convulsions and was often fatal. Survivors suffered a number of neurological complications such as mental retardation, deafness, blindness, and convulsions (*1*). The recognition of progressive loss of intellectual ability in sibs of those clinically affected, but who had lower levels of lead exposure, contributed to the concept of subclinical or asymptomatic CNS effects. In the 1974 NIEHS symposium, three studies were presented that were concerned with neuropsychological outcomes in children with PbB levels below 40 μg/dL (*5*). In the mid-1970s, De la Burde and Choate (*25*) found impairment of fine motor function, concept formation, and altered behavioral profiles in 70 preschool children with elevated PbB and history of pica compared to controls. PbB levels were greater than 30 μg/dL in all cases; the mean was 59 μg/dL. In a follow-up study in 1975 on the same children at 7 and 8 years old, De la Burde and Choate found continued CNS impairment as assessed by a variety of psychological and neurological tests (*26*).

In a similar type of study in 1979, Rummo and colleagues (*27*) found significant neurobehavioral deficits including hyperactivity and lower scores on McCarthy scales of cognitive function among Providence, Rhode Island, inner-city children who had previously experienced high lead exposure that had produced encephalopathy. The mean maximum PbB level at the time of encephalopathy was 88 ± 40 μg/dL. However, children with PbB lower than the maximum mean did not differ from controls on these tests. Review of these data by Grant and Davis (*28*) suggested that the failure to find significant differences in this latter group was due to small sample size. The results of these and similar studies are also summarized by the U.S. EPA (*19*) and in the published proceedings of a conference on Lead Exposure and Child Development, with the conclusion that CNS effects in infants and young children occur with PbB levels in the 10–15 μg/dL range (*28*). The major methodological problems in these studies concern confounding factors and limitations in statistical power for identifying such a small effect. Also, studies with cross-sectional design have problems with estimates of exposure.

To increase the analytical power of studies reported to date, Needleman and Gatsonis (*29*) performed a meta-analysis on 12 previously performed cross-sectional epidemiological studies relating children's I.Q. to PbB levels at low doses. Each of the studies controlled nonlead factors or covariates. The results supported the hypothesis that lead impairs children's I.Q. at low doses. However, meta-analysis of independent studies have been subject to criticism. They may introduce bias in that they use only published information, whereas papers with insignificant findings tend to remain unpublished. Also, the mix of studies are heterogenous in terms of design, methods of lead analysis, confounder structure, as well as overall statistical analysis.

To increase sample size, the World Health Organization, Regional Office for Europe (WHO/Euro) in collaboration with the Commission of the European Communities conducted a combined study involving eight groups from eight countries [Bulgaria, Denmark, Greece, Hungary, Italy, Rumania, West Germany, and Yugoslavia (*30*)]. The study followed a common protocol with quality assurance measures to achieve comparability. PbB concentration was the main marker of exposure. The Weschler Intelligence Scale for Children (WISC) (four subtests) for psychometric intelligence, the Bonder Gestalt test (GFT version) and the Trail-Making test for visual motor integration, the Vienna Reaction Device and delayed reaction time task for reaction performance, and the Needleman scales for behavior ratings served as behavioral end points. The combined individual studies represented a sample size of 1879 school-age children and covered a PbB range from below 5 μg to about 60 μg/dL. The combined analysis of the full set of data across studies demonstrated a significant linear relationship between PbB and visual-motor integration and serial choice reaction performance. These results were largely consistent with previous cross-sectional studies. Psychometric intelligence as assessed by means of the WISC was also affected by lead exposure, but the overall degree of association was of only borderline significance and did not fit as smoothly into the overall pattern of findings as determined in the Needleman and Gatsonis meta-analysis study (*29*). The authors also point out that the full data set of this large combined study did not permit the identification of an effect threshold, that is, a biological exposure limit above which adverse health effects are to be expected (*30*).

To overcome the difficulties inherent in cross-sectional studies a number of prospective studies have been instituted. Results from four such studies conducted in Boston (*31,32*), Cincinnati (*33*), Port Pirie, Australia (*34*), and Cleveland (*35*) are becoming available.

Bellinger and colleagues (*31*) are conducting a longitudinal study of early neurobehavioral development through the first 2 years of life in a cohort of 170 upper and upper-middle class Boston children. This group has shown that performance on the Bayley Mental Development Index (MDI) at 6, 12, 18, and 24 months postnatally is inversely related to PbB lead levels at birth and that the amount of deficit in infants with cord PbB levels of 10–25 μg/dL is approximately 4–8 points on the MDI. The MDI effect was evident across the entire range of PbB levels starting at 10 g/dL. The mean PbB at age 24 months was 6.8 μg/dL (SD = 6.3; 75th, 90th, and 99th percentiles: 8.8, 13.7, 23.6 μg/dL). After adjusting for confounding, scores on the general cognitive index decreased approximately 3 points (SE = 1.4) for each natural log unit increase in 24-month PbB level.

By 5 years of age, the association between prenatal lead exposure and children's cognitive function diminished greatly in the cohort as a whole, except among children with higher concurrent lead exposure risk (*32*). Improvement was associated with lower PbB levels at 57 months of age. The data suggest a socioeconomic strata (SES) effect in that the second year MDI performance of lower SES children was more adversely affected at lower PbB levels (6–7 μg/dL) than was the performance of higher SES children. These investigators found improvement in cognitive performance in children followed from 24 to 57 months of age. Improvement was associated with lower PbB levels at 57 months of age. Risk of persistence of cognitive deficit was increased in children with higher postnatal exposure and less favorable SES factors. Higher prenatal exposures were not associated with lower scores at 57 months, except in the subgroups of children with "high" concurrent PbB levels (i.e., ≥ 10 μg/dL).

Dietrich et al. (*33*) have reported interim results for neuro-

behavioral outcomes in about 300 infants from a study of inner city infants born in Cincinnati, Ohio. The children were tested on the Bayley Scales at 3, 6, 12, and 24 months of age. After adjusting for covariates, deficits in 3- and 6-month Bayley MDI scores were significantly associated with prenatal (maternal) and cord PbB levels. The MDI deficits at 3 months were about 6 points for every 10 μg/dL increment in cord PbB and at 6 months, the MDI deficit was about 7 points per 10 μg/dL increment in cord PbB. However, neither prenatal nor cord PbB levels were significantly related to the 12-month MDI scores, and by 24 months there was no statistically significant relationship between lead exposure and MDI scores. Deitrich et al. interpreted the failure to detect a persistent effect of fetal lead exposure on the 24 month Bayley Scales as probably due to a neurobehavioral "catch-up response" analogous to that observed in infant twins and other twins compromised during prenatal development (*33*).

In a prospective study of more than 500 children in Port Pirie, Australia, McMichael et al. (*34*) found a highly significant inverse association between average prenatal PbB level and cognitive development in 4 year olds. Port Pirie is located near a lead smelter. Samples for measuring PbB levels were obtained from mothers antenatally, at delivery, from mothers and umbilical cords and at ages of 6, 15, and 24 months and then annually. Concurrently, the mothers were interviewed about personal, family, medical, and environmental factors. Maternal intelligence, the home environment, and the children's mental development as evaluated with use of the McCarthy Scales of Children's abilities were formally assessed. Mean PbB went from 0.44 μmole/L (9 μg/dL) in mid-pregnancy to a peak of 1.03 μmole/L (21 μg/dL) at age 2 years. Children with an average postnatal PbB level of 1.50 μmole/L (30 μg/dL) had a general cognitive index 7.2 points lower than those with average concentration of 0.5 μmole/L (10 μg/dL). No threshold dose for the lead effect was evident. However, these associations were greatly reduced after entering a large number of social, demographic, and biomedical covariates in the regression model. The authors suggest caution in making causal inferences from these studies because of the difficulties in defining and controlling the confounding effects.

Ernhart and colleagues in Cleveland (*35*) followed a cohort of 242 children initially identified at the mother's first antenatal visit to clinics that serve the indigent population of Cleveland. By selection, half of the mothers had histories of alcohol abuse. The others were matched controls. By 4 years, 10 months, 260 children remained in the cohort. PbB was collected through age 3 years and related to a series of development tests. Ernhart et al. found statistically significant correlations between most of the PbB measurements and the intelligence test scores (*34*). However, they found that changes were not consistent in direction when relevant confounding variables were considered and concluded that the findings did not provide clear and consistent support for a relationship between pre- and postnatal PbB and results of developmental tests through age 3 years. Small sample size and the fact that half of the samples were composed of alcoholic pregnancies may have limited this study and ability to detect an effect of lead, if present.

In addition to these studies, reports are now emerging from a number of other prospective studies on effects of lead in exposure during gestation and early childhood. These studies include populations from Sydney, Australia; Mexico City; Yugoslavia; Glasgow, Scotland; Christchurch, New Zealand; and Nordenham, Germany. Rothenberg and colleagues (*36*) in Mexico City have reported effects of maternal PbB at 36 weeks and birth and of umbilical cord PbB on neurobehavioral development in the first 30 days of life. They observed that a lower PbB level at 36 weeks of pregnancy relative to maternal PbB level at birth was a significant predicator of abnormal reflex as measured by the Brazelton Neurobehavioral Assessment Scale. Mean PbB of mothers was 15.0 μg/dL at 36 weeks and 15.0 μg/dL at birth. The PbB level of infants at birth was 13.8 μg/dL.

Taken together, the above studies demonstrate that lead has an adverse health effect on neurobehavioral and cognitive development at PbB levels in the 10–15 μg/dL range in infants, children, and the fetus, and perhaps at even lower levels. The magnitude of I.Q. decrements are dose related, as shown in Figure 1.

The importance of a 4–7 point reduction in I.Q. may at first appear unimportant, but further analyses suggest that small changes are of considerable significance. When the actual cumulative frequency distribution of I.Q. between high- and low-lead subjects are plotted and compared, there is a nearly 4-fold increase in the number of children with a severe deficit, that is, I.Q. scores below 80. There is also a 5% reduction in the number of children with superior function (I.Q. > 125). There is currently no estimate of the cost of this effect to society in social or monetary terms.

Reversibility of CNS Effects

There is now question as to whether the small changes in behavioral and cognitive development observed in recent studies is reversible. The two prospective studies from Boston (*37*) and Cincinnati (*33*) of cognitive effects of neonatal exposure to lead suggest some reversal may occur if PbB levels decline during early childhood and SES is favorable. However, Needleman et al. (*38*) found a decrease in neurobehavioral function persistent into adolescence in a group with higher PbB levels (> 30 μg/dL). They followed 132 young adults initially studied as primary schoolchildren in 1979. At that time neurobehavioral function was inversely related to dentine lead levels; the children were divided into two groups based on dentine lead levels. The high-level group had an average PbB level of 35.5 μg/dL; the low-level group had an average PbB level of 23.8 μg/dL. Needleman et al. found that lead content of teeth shed at ages 6 and 7 was associated with an increase in dropout rate from high school and reading disability (*38*). Higher lead levels were also associated with lower class standing, increased absenteeism, lower vocabulary and grammatical reasoning scores, poorer hand–eye coordination, longer reaction time, and slower finger tapping. Needleman et al. concluded that PbB levels in childhood >30 μg/dL are associated with deficits in central nervous system function that persist into young adulthood (*38*).

Mechanisms of CNS Effects

Twenty years or more ago, knowledge of mechanisms of lead toxicity was largely based on autopsy findings of children who died with lead encephalopathy. The major findings were a severe edema of the brain related to an increase in proteinaceous transudate from permeable capillaries and intercapillary spaces (*1*). An example of this phenomenon is shown in Figure 2. Although

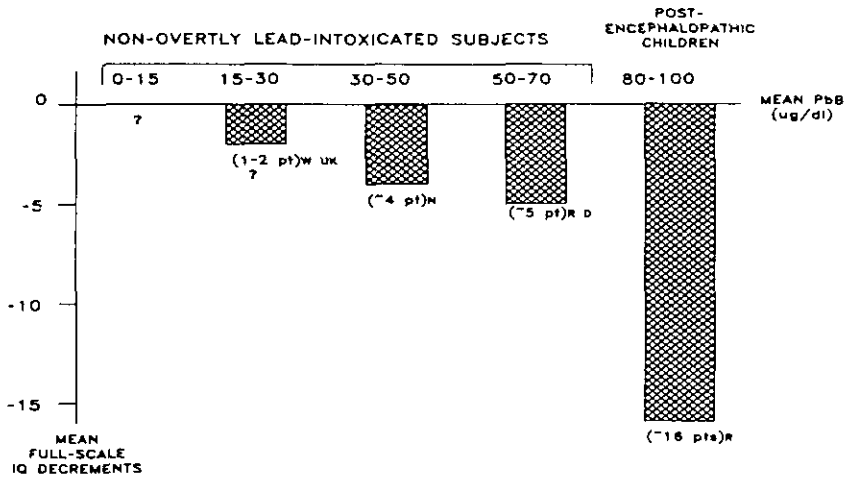

FIGURE 1. General pattern of results from cross-sectional population studies of exposure effects on cognitive function as reflected by deficits in I.Q. scores on various standardized tests. The magnitude of I.Q. decrements associated with a broad range of blood lead (PbB) levels is estimated here based on evaluation of studies noted and commented on in text. More recent prospective studies identify lead effects on I.Q. from PbB levels as low as 10–15 μg/dL or perhaps lower. R, Rummo studies (*27*); D, de la Burde studies (*25,26*); N, Needleman studies (*29*); W, Winneke studies (*30*); UK, studies by Smith et al. (*39*). This figure is adapted with permission from Grant and Davis (*28*).

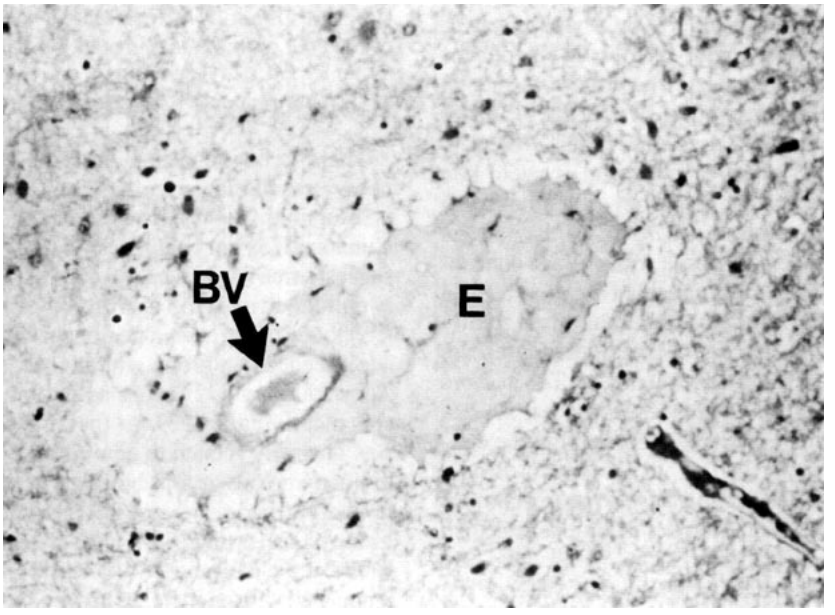

FIGURE 2. Photomicrograph of section of cerebral cortex taken at autopsy of a child dying of acute lead encephalopathy showing blood vessel (BV) surrounded by lead-containing edema fluid. Pathological effects of lead are a consequence of toxicity to neurons and neurotransmitters and accompanying increase in intracerebral pressure edema. E, edema (transudate). Photomicrograph is provided through the courtesy of David Munoz (Neuropathology, University Hospital, London, Ontario).

the mechanisms by which lead disrupts brain function and the reasons for the apparent increase in sensitivity of the immature brain to lead toxicity are not yet completely understood, our knowledge has increased considerably. This new information is contained in many publications including articles from an NIEHS conference on Advances in Lead Research published in *EHP* (*12*).

It is suggested that the immature endothelial cells forming the capillaries of the developing brain are less resistant to the effects of lead than capillaries from mature brains. These cells permit fluid and cations including lead to reach newly formed components of the brain, particularly astrocytes and neurones (*40*). Markovac and Goldstein (*41*) investigated the effects of inorganic lead on calcium, phospholipid-dependent protein kinase (protein kinase C) in brain microvessels from 6-day-old rat pups. They found that micromolar concentrations of lead activate this enzyme to an extent equivalent to micromolar calcium, suggesting this may be a sensitive mechanism for lead cellular toxicity in

capillaries by resulting in a breakdown in the normally tight blood–brain barrier.

Exposure of the developing fetus to lead may result in higher brain accumulation than postnatal exposure. Rossouw et al. (*42*) compared brain lead levels following a 3-week postnatal exposure, either preweaning (days 1–21) or post-weaning (days 22–43) and exposure of the fetus via maternal administration. The exposure *in utero* resulted in a 6-fold increase in total brain lead (1.07 μg/g) versus 3.5- and 2-fold increases at the other time points (0.84 μg/g and 0.44 μg/g). Exposures at these three different developmental phases also resulted in differential effects on monamine and cholinergic receptors. Significant differences were found in either suckling or fetal rats with no changes after post-weaning exposure.

Neurochemical studies in experimental models have shown that lead, in the absence of morphologic changes, produces deficits in neurotransmission through inhibition of cholinergic function, possibly by reduction of extracellular calcium. Other noted changes in neurotransmitter function include impairment of dopamine uptake by synaptosomes and impairment of the function of the inhibitory neurotransmitter α-aminobutyric acid (*18*).

There is also some experimental evidence to suggest that the fetal brain may have greater sensitivity to lead than the mature brain. Rat pups readily develop encephalopathy during the first few days of life, and resistance to lead-induced neurotoxicity occurs after 18–20 days of age. The development of resistance to lead encephalopathy is thought by Holtzman and co-workers (*43*) to be related to the formation of lead–protein complexes (inclusion bodies) in astrocytes that sequester lead away from mitochondria. This role of lead–protein complexing is similar to that postulated in renal tubular epithelial cells as a mechanism for permitting the kidney to excrete lead without cellular toxicity (*43*). The lead protein complexes are thought to contain a number of richly acidic proteins (*44,35*). More recently, Egle and Shelton (*46*) have identified the most abundant protein component of isolated inclusion bodies to be a constitutive protein of the central nervous system, most abundant in the cerebral cortex. In rat brain, the protein was found to be developmentally regulated. Only traces were detected 3 days after birth, but within 1–2 weeks adult levels were achieved. These studies suggest, therefore, that this cellular mechanism to sequester lead is not present in the fetal brain.

Cardiovascular Disease

Effects of lead on cardiovascular disease have been suspected for many years. However, the evidence 20 years ago was largely negative apart from a few conflicting research reports (*1*). Epidemiological studies of lead effect on blood pressure in occupationally exposed workers showed essentially no effect. The reasoning followed that if heavily exposed workers were not affected, it was not likely that lesser exposed people in the general population would be adversely affected. Nevertheless, analysis of the Second Natonal Health and Nutrition Examination Survey data (*47*) showed a significant linear association between PbB concentrations and blood pressure. Additional analysis of the data by Pirkle et al. (*48*) focused on white males aged 40–59. In the range of 7–34 μg/dL, there was no evident threshold below which PbB level was not significantly related to blood pressure. The dose–response relationship indicated that large initial increments in blood pressure occur at relatively low PbB levels, followed by leveling off of blood pressure at higher PbB levels. This may explain why a blood pressure effect was not previously identified in a lead-exposed worker population. However, a lead effect on blood pressure on workers with low level exposure has been more recently demonstrated. Sharp et al. (*49*) reported a relationship between lead exposure and elevated blood pressure in San Francisco bus drivers examined using multiple regression analysis. Systolic and diastolic pressures varied from 102 to 173 mmHg and from 61 to 105 mmHg, respectively. The PbB concentration varied from 2 to 15 μg/dL. Effect on blood pressure is now identified as the critical effect of lead in adults (*19*).

The mechanism whereby lead affects blood pressure is not known, although experimental studies have suggested several plausible possibilities. Much of this is contained in an *EHP* publication of Proceedings of a 1988 Symposium on Lead–Blood Pressure Relationships (*11*) and a recent review by Schwartz (*50*). Two major mechanisms of action are an effect of lead on the calcium-mediated control of vascular smooth muscle contraction and renal effects mediated through renin–angiotensin system. The work of several investigators presented at the 1988 symposium suggest that lead increases pressor responsiveness to catecholamines, which may be a consequence of the lead effect on the intercellular messenger protein kinase C and its role in smooth muscle contraction (*51*). The human and experimental data on lead effect on the renin–angiotensin system is not clear with findings of both increased and decreased plasma renin activity following exposure. Vander (*52*) suggests from experimental studies on rats and dogs that there is an initial increase in plasma renin activity followed by gradual reduction with chronic exposure.

Bone

Metabolic Effects

Lead in bone is of interest for two reasons. Bone is the largest repository of the body burden of lead, and, secondly, it is now recognized that lead may, in fact, have an effect on bone metabolism. Of current concern is that lead in bone may be mobilized during a number of physiological and pathological conditions such as age, endocrine status, osteoporosis, renal disease and, in particular, during pregnancy and lactation. Silbergeld (*53*) has summarized a number of important factors in determining the extent to which bone lead may be mobilized during gestation. Obviously, the most important of these factors is the level of lead in bone. Rabinowitz (*54*) has suggested that the dose rate of lead exposure influences location and concentration of lead in different sites in bone, which may influence its availability for mobilization. Other factors are maternal age, gestational age, parity, and race. The major determinant for each of these factors is nutritional status, particularly dietary calcium.

Pounds and co-workers (*55*) have reviewed the possible mechanisms whereby lead may directly or indirectly alter several aspects of bone cell function. Clinical studies have shown that exposure to lead may change circulating levels of 1,25-dihydroxyvitamin D_3. Secondly, lead may affect the ability of bone cells to respond to hormone regulation. A calcium-binding protein, osteocalcin, synthesized by osteoblasts, is inhibited at low levels

of lead exposure, which may impair new bone formation as well as the functional coupling of osteoblasts and osteoclasts. Lead may also impair synthesis of components of bone matrix such as collagen or bone sialoproteins. A common molecular basis for the cellular effects may be altered or impaired calcium and cAMP messenger systems in cells.

Measurement of Lead in Bone

There is increasing interest in the development of analytical systems based on the principle of X-ray fluorescence (XRF) for the *in vivo* measurement of lead in bone. Three basic designs have been reported in the literature and summarized by Thomas (*56*). Two of these use excitation and detection of K X-rays of lead; the third uses excitation and detection of L X-rays. The major difference in the K and L systems is the photon source that is used, either ^{109}Cd or ^{57}Co. Both types of systems using detection of K and L X-rays involve relatively low energy incident and emitted photons and are therefore limited to measuring lead in superficial bone. L X-ray systems have been applied only to measuring the cortical region of the tibia where overlying tissue is thin in order to have maximum penetration. Depth of measurement is approximately 2.8 mm. Sensitivity decreases to 30% at approximately 1.3 mm. Sensitivity of systems using K X-rays, however, have a 30% reduction in sensitivity at sites approximately 25 mm from the surface. This system, therefore, has the potential for use at a number of bone sites, but has been used for primarily measuring lead in sections of the phalanx or tibia. Whether results of these measurements can be used to assess total body burden of lead is currently uncertain. The major limiting factor is the limit of sensitivity for lead in the common range of 10–20 ppm. Rosen et al. (*57*) have performed sequential measurements of bone lead in $CaNa_2$ EDTA-treated lead-toxic children using L X-ray fluorescence. In this system the minimum detection limit was 7 μg of lead/g of bone. Cortical bone lead values were similar to lead concentrations measured via bone biopsy in normal adults and lead workers and were a predictor of $CaNA_2$ EDTA treatment outcomes in 86% of lead toxic children (PbB of 25–55 μg/dL) compared to a 93% result using PbB as a predictor. A question is whether the specificity and sensitivity of this system can be extended to measure bone lead levels present in children with minimal or low-level lead exposure.

Reproductive Effects

It has been recognized for decades that severe lead intoxication is associated with sterility, abortion, stillbirths, and neonatal morbidity and mortality from exposure *in utero* (*1*), but the potential for effects of low-level lead exposure on reproductive fitness in males and females is only beginning to receive the attention of experimental and clinical toxicologists. In a recent conference on Advances in Lead Research reported in *EHP* in 1990, several presentations focused on effects on the fetus *in utero*, but little new information was available regarding effects on reproduction (*12*). Nevertheless, there are indications of developing interest.

In a recent study performed in Yugoslavia, semen quality from 152 male volunteers with no occupational exposure to lead or cadmium was compared to 101 workers with slight to excessive occupational exposure to lead (*58*). Median PbB of control workers was 10.7 μg/dL (range 6.7–20.8 μg/dL) and exposed workers 37.1 μg/dL (range 11.7–104.0 μg/dL). Increase in PbB reduces semen volume, semen density, counts of total, motile, and viable sperm, percentage of progressively motile sperm, levels of zinc, acid phosphatase, and citric acid, and increases the percentage of pathological sperm. These data suggest that male reproductive effects may also occur at PbB levels at the 30–40 μg/dL range or even lower.

There are no recent studies on effects of lead on reproductive function in women, although it has been known for many years that lead may be an abortifacient when women are acutely exposed to high doses. A recent study conducted by Health and Welfare, Canada (*59*) on female nulliparous monkeys has indicated that chronic exposure to lead with PbB levels of approximately 35 μg/dL resulted in subclinical suppression of circulating luteinizing and follicle-stimulating hormone and estradiol without overt effects on general health and menstruation.

Nephrotoxicity and Carcinogenesis

Lead Nephropathy

Lead nephropathy in rodents progresses to renal adenocarcinoma. However, a similar relationship between nephropathy in workers with occupational exposure to lead and renal cancer has not been clearly established from epidemiological studies, although two case reports have been published (*60,61*). The potential carcinogenesis of lead has been reviewed recently by EPA because of the uncertainty of this relationship (*62*). Lead effects on the kidney have provided one of the oldest and most-studied experimental models of cellular effects of lead. Clinically important renal effects of lead are uncommon today and effects of low-level exposure on renal function and even carcinogenesis is not known. The comments in this article are largely taken with permission from a presentation I gave at a Society of Toxicology symposium in 1991, which was recently published (*63*).

Overt effects of lead on the kidney in man and experimental animals, particularly the rat and mouse, begin with acute morphologic changes consisting of nuclear inclusion bodies or lead–protein complexes and ultrastructural changes in organelles, particularly mitochondria. Progression of acute nephropathy to chronic irreversible nephropathy occurs slowly, over months or years, and only after years of heavy exposure.

Selevan et al. (*64*) reviewed the patterns of death for a cohort of 1987 male smelter workers employed between 1940 and 1965. Although overall mortality was similar to that of the U.S. white male population, there was an excess mortality from chronic renal disease. The risk of death from renal disease increased with increasing duration of employment. In a mortality study of 4519 battery plant workers and 2300 lead production workers by Cooper and co-workers (*65*), there was excess mortality from chronic nephritis. The mean PbB concentration of 1326 of the battery workers in this study with three or more analyses was 62.7 μg/100 g, 404 had means of 70 μg or more, 24 of which had means over 100 μg/100 g. For the lead production workers in four plants, the mean for 537 men was 79.7 μg/100 g. These workers were therefore exposed to lead in amounts far exceeding present workplace standards.

Experimental studies suggest that there may be a threshold for lead nephrotoxicity. Rats exposed to different doses of lead for

up to 12 weeks suggest that PbB level of 60 μg/dL is the threshold for proximal tubular cell injury from lead (*66*). This level of PbB is equivalent to kidney lead of about 45 g/g and is the level of lead at which renal calcium increases and ultrastructural changes in proximal tubular cell mitochondria occur. This finding is consistent with the observation of Buchet et al. (*67*) that workers who do not have a PbB level over 62 or 63 μg/dL for up to 12 years do not have lead nephropathy.

Renal biopsies of a small group of shipwreckers with heavy exposure to lead has shown that histologic features of early and chronic exposure to lead is similar in experimental animals and man, suggesting similar pathogenetic mechanisms (*68*). Nuclear inclusion bodies become less common as renal tubular atrophy and interstitial fibrosis increase in severity. Proximal tubular dysfunction is usually not demonstrable in the chronic phase of lead nephropathy, but interstitial fibrosis is usually associated with asymptomatic renal azotemia and reduced glomerular filtration rate. In workers without azotemia but decreased insulin clearance, there is decreased maximum reabsorption of glucose. Although progression from acute, reversible to chronic, irreversible lead nephropathy has not been clearly shown to occur in man, this progression has been demonstrated in rodent models.

Lead and Cancer

There are several experimental studies in rats or mice in which long-term administration of a lead compound in food or drinking water produced renal tumors. The studies have been summarized by the EPA (*62*) and in earlier reviews (*69*). The studies show that renal carcinogenicity occurs on a background of proximal tubular cell hyperplasia, cytomegaly and cellular dysplasia. Renal adenocarcinoma occurs in a high percentage of exposed animals, and incidence is dependent on length and severity of exposure (*70,71*).

Males are more susceptible to tumors than females. Tumors have not been shown to occur below the maximum tolerated dose (*72,73*) of 200 ppm of lead in drinking water. This is the maximum dose of lead in rats not associated with any morphologic or functional evidence of renal toxicity in rats fed a diet containing adequate levels of essential minerals, particularly calcium (*74*).

Epidemiologic studies of workmen exposed to lead in a number of different occupational settings have been reported (*62*). Most of the studies are proportional mortality studies and contain numerous deficiencies, particularly lack of information on cumulative lead exposure. A nonsignificant excess of kidney cancer was observed in a cohort mortality study of primary lead smelter workers (*64*), but this was based on only six cases. Cantor et al. (*75*) found a borderline significant excess of kidney cancer in a study of 7121 members and retirees of the United Association of Plumbers and Pipe Fitters in California. In addition, there have been two case reports of kidney cancer among lead workers (*60,61*). Excess lung cancers were observed in several studies, but these cannot be attributed to lead because of confounding exposures to other heavy metals, including arsenic and chromium and to smoking cigarettes. Also, in each study the presence of other potentially carcinogenic agents in the workplace make it difficult to determine if any increase in risk is due to lead per se or possibly other agents. Nevertheless, the studies overall do not provide conclusive evidence of an association of lead exposure and an increased incidence of cancer (*62,76*).

Mechanisms for Lead Carcinogenesis

Table 1 is a short list of possible mechanisms whereby lead may induce renal tumors. The following brief comments are provided regarding each of the proposed mechanisms. The mechanisms are not mutually exclusive, nor is supportive evidence complete in any case.

Lead is thought to have genotoxic properties, but lead-induced gene mutations in cultured mammalian cells have only been observed at toxic concentrations. Studies for point mutations in bacterial systems have been negative, but these systems are usually negative and may be inappropriate for assaying metal ions. However, Zelikoff and co-workers (*77*) found that both insoluble lead sulfide and more soluble lead nitrate were mutagenic when added to Chinese hamster V79 cells. They also found that lead acetate induces morphological transformation in Syrian hamster cells. However, they conclude that these effects may not be the result of direct damage to DNA, but may occur via indirect mechanisms including disturbances in enzyme functions important in DNA synthesis and/or repair or in DNA helical structure. These effects may also be influenced by synergistic factors such as calcium deficiency.

The occurrence of nuclear inclusion bodies or lead–protein complexes in proximal tubular cells of lead-exposed animals and humans has prompted speculation that the nonhistone proteins involved in the formation of these bodies alters genetic function in some way, but there is no definitive basis for this assumption.

The observation that picomolar concentrations of lead acetate can activate partially purified protein kinase C from rat brain suggests that lead may be acting as a co-carcinogen or tumor promoter (*41*). This enzyme is regulated by availability of both calcium and diacylglycerol, is activated by a number of known tumor promoters, and is thought to be the primary cellular target for the phorbol esters. The activated enzyme phosphorylates a number of cellular protein substrates including growth factor receptors and oncogenes.

There is also *in vivo* evidence that lead may act as a tumor promoter. Lead increases lung tumor incidence and lung tumors per mouse in a mouse lung adenoma model (*78*) and increases cerebral gliomas in rats (*79*). Addition of lead acetate to the diets of rats exposed to N-(4-fluorobiphenyl)4-acetamide, a known carcinogen, increases renal tumor to 100% suggesting that lead may be a promoter (*80*).

Lead is a mitogen. Exposure to a single IP injection stimulates a 40-fold increase in cell proliferation in rats as measured by autoradiography and is further increased by unilateral nephrectomy (*81*). DNA synthesis in kidneys as measured by ^{3}H-thymidine incorporation is increased 15-fold and mitotic index 45-fold following a single intracardiac injection of lead acetate

Table 1. Possible mechanisms relating renal carcinogenesis to lead-induced chronic nephrotoxicity.

Mutagenicity
Nuclear protein effect (inclusion bodies)
Promoter activity, activation of protein kinase C
Cellular proliferation
Cystic hyperplasia

in mice (*82*). More recently, cell proliferation and hyperplasia were seen in the liver of rats given a single IV injection of lead nitrate (*83*). In both of these models, increases in cell proliferation occurred in the absence of cellular necrosis, suggesting this is a mitogenic response rather than a regenerative response.

Cystic hyperplasia is a late morphologic feature of chronic lead nephropathy in humans and experimental animals and imposes an increased risk of malignancy (*84*). Cystic hyperplasia has been found to be a morphologic precursor to adenoma formation in animals treated with renal carcinogens, nitrolotriacetic acid (*85*), in rats exposed to *N*-(4-fluoro-4-biphenyl)acetamide (*86*), and in persons with hereditary adult polycystic kidney disease (*87*) and acquired renal cystic disease occurring in patients with chronic renal failure on hemodialysis (*88*). One of the proposed hypotheses relating disordered epithelial cell growth to renal cyst formation suggests that cells lining cysts become transformed and proliferate abnormally, presumably in response to increased intracystic fluid volumes. Regardless of the mechanism, the available literature from both human and experimental studies suggest that renal cyst formation as occurs in chronic renal failure induced by lead or from a variety of other causes, genetic or acquired, contributes to an increase in renal adenocarcinomas. In such cases, the adenocarcinoma is a consequence of the cystic change in the renal cortex that follows chronic lead nephropathy.

Major Risk Factors for Lead Toxicity

In the second issue of *EHP* about 20 years ago, an article on susceptibility to lead toxicity reviewed a number of factors that influence risk to lead toxicity (*89*). Many of the issues discussed then are relevant today, but research over the past 20 years has shown some to be of greater significance than others. Table 2 identifies what are currently the major risk factors. It is important to identify such factors for the management of the lead toxicity problem because control of the factors provide the greatest opportunity for targeting primary prevention and intervention strategies.

Age

Infants and young children have long been known to be at increased risk to toxicity to lead because of higher lead intake relative to body size and greater absorption from the gastrointestinal tract. The central nervous system of the developing fetus may be at even greater risk because of immaturity of the blood–brain barrier.

The ratio of PbB to tissue organ lead also changes with age. The rapid growth rate during the first few months of life results in declining lead dose in terms of absolute intake per body weight. The fraction of lead in bone also increases with age. Barry has shown that 73% of body lead is in bone during childhood, increasing to approximately 95% in adult life (*89,90*).

Table 2. Major risk factors for lead toxicity.

Age
Nutrition (food intake)
Essential metals
Calcium, iron, zinc
Housing and socioeconomic status

Nutrition

Mahaffey has summarized the influence of total food intake and patterns of food intake on lead toxicity (*91*). Several observations have been made. Lead intake from water and other beverages is absorbed to a greater degree than lead in food. Lead ingested between meals is more greatly absorbed than lead with meals, and increasing frequency of food intake minimizes lead absorption. The composition of the diet, particularly high protein content, was shown in experimental studies many years ago to decrease lead intake, but there has been less interest or new information regarding this factor in recent years. Lead interacts or competes for gastrointestinal absorption, tissue deposition, and as a co-factor in metallo-enzymes with essential trace metals, particularly calcium, iron, and zinc. Deficiencies of calcium and iron clearly enhance lead absorption. It has been recognized for many years that lead metabolism mimics calcium metabolism in many ways. It is also clear from animal studies that calcium deficiency greatly enhances lead absorption and that periods of low calcium mobilize lead from tissue stores, particularly bone. This is particularly true for fetal growth *in utero* where increase in fetal tissue lead parallels increase in tissue calcium (*92*). This phenomenon also occurs during lactation where lead appears along with calcium in mothers' milk (*93*). A topic of speculation with little evidence to date is to what degree calcium excess beyond recognized daily requirements diminishes lead uptake and tissue toxicity.

Lead competes with zinc as a co-factor with heme enzymes, particularly aminolevulinic acid dehydratase and ferrochelatase. Iron deficiency increases erythrocyte protoporphyrin activity as do increasing PbB levels, probably not because of co-factor competition but because both metals stimulate precursor synthesis. One of the most provocative observations, reported by Oski et al. (*94*), is that iron therapy improves developmental scores of infants. The molecular basis for this is unknown and remains an area needing further clarification. Increasing zinc in the diet reduces tissue accumulation and toxicity of lead (*95*). This effect is thought to be due to enhanced gastrointestinal absorption of lead because zinc deficiency does not enhance toxicity of parenteral lead.

Housing and Socioeconomic Status

It has been recognized for several decades that children living in older housing containing paint with high lead content are at high risk for excess lead exposure and toxicity. An in-depth analysis of this problem is contained in a 1988 report from the Agency for Toxic Substances and Disease Registry (*96*). This analysis concludes that about 2.4 million white and black metropolitan children, or about 17% of such children in the U.S. metropolitan areas, are exposed to environmental sources of lead at concentrations that place them at risk to lead toxicity. The number approaches 3 million black and white children if extended to the entire U.S. population, and may be as many as 4 million children if children from other racial groups (Hispanic, Oriental, and other origins) are included. Of these children, about 5% have PbB levels greater than 20 μg/dL. Black, inner-city, or low-income strata have a much higher percentage with elevated PbB levels, although no economic or racial subgroup is exempt. Furthermore, an estimated 400,000 fetuses *in utero* are exposed to

maternal PbB levels greater than 10 μg/dL and are therefore also at risk to lead effects.

Other major environmental sources of lead in addition to paint are lead in dust, soil, food, and water (*18*). Lead levels in dust and soil result from past and present inputs from paint, air fallout from paint emissions, and lead in gasoline. While lead in paint, dust, and soil are the major environmental sources of exposure, lead in drinking water provides a significant risk, particularly as an additive factor. Furthermore, the older the housing, the more likely there may be lead piping. Reduction of lead in gasoline has reduced atmospheric lead fallout deposited in water, dust, and soil and has probably contributed to reduction of lead in food. Elimination of the use of lead solder in canned food has also contributed to reduced lead in food. Continued progress in these areas will play a major role in diminishing or even eliminating the risks to lead toxicity.

The Future

In a statement titled "Promoting Health/Preventing Disease: Year 2000, Objectives for the Nation," the Public Health Service goal is to "reduce the prevalence of blood lead levels exceeding 15 μg/dL and 25 μg/dL among children ages 6 months through 5 years to no more than 500,000 and zero" (*97*). A special population target is inner city black children (annual family income of less than $6000 in 1984) ages 6 months through 5 years. To achieve this goal, the Department of Health and Human Services and the Department of Housing and Urban Development and the Environmental Protection Agency have released a strategic plan for the reduction of lead poisoning. Major components of the plan are prevention activities, increased abatement of environmental exposures, principally housing with lead containing paint, and national surveillance programs essential to identifying progress and children at risk.

Also essential to the plan is increasing research to understand the health hazards caused by exposure to lead. Many of the studies, particularly the basic research contained in this report, have been supported by the extramural program of the NIEHS. Currently more than $8 million per annum is directed to lead research from NIESH. It is hoped that we can address many of the current concerns outlined here in the future.

REFERENCES

1. Goyer, R. A., and Rhyne, B. C. Pathological effects of lead. Int. Rev. Exp. Pathol. 12: 1-77 (1973).
2. NAS/NRC. Airborne lead in perspective. National Academy of Sciences, Washington, DC. 1972.
3. WHO. Environmental Health Criteria 3. Lead. World Health Organization, Geneva, 1977.
4. Low-Level Lead Toxicity. Environ. Health Perspect. 7 (1974).
5. Heavy Metals in the Environment. Environ. Health Perspect. 12 (1975).
6. Environmental Lead and Arsenic. Environ. Health Perspect. 19 (1977).
7. Factors Influencing Metal Toxicity. Environ. Health Perspect. 25 (1978).
8. Role of Metals in Carcinogenicity. Environ. Health Perspect. 40 (1981).
9. Health Effects of Toxic Wastes. Environ. Health Perspect. 48 (1983).
10. Health Effects of Acid Precipitation. Environ. Health Perspect. 63 (1985).
11. Lead-Blood Pressure Relationships. Environ. Health Perspect. 78 (1988).
12. Advances in Lead Research. Environ. Health Perspect. 89 (1990).
13. Lead in Bone. Environ. Health Perspect. 91 (1991).
14. Steinfeld, J. L. Medical aspects of childhood lead poisoning. Pediatrics 48: 464-468 (1971).
15. CDC. Increase Lead Absorption and Lead Poisoning in Young Children: A Statement by the Centers for Disease Control. U.S. Department of Health Education and Welfare, Atlanta, GA, 1975.
16. CDC. Preventing Lead Poisoning in Young Children: A Statement by the Centers for Disease Control. U.S. Department of Health and Human Services, Atlanta, GA, 1985.
17. CDC. Preventing Lead Poisoning in Young Children: A Statement by the Centers for Disease Control. U.S. Department of Health and Human Services, Atlanta, GA, 1991.
18. EPA. Air Quality Criteria for Lead, Vol. 1-4. EPA-600/8-83/028aF, U.S. Environmental Protection Agency, Environmental Criteria and Assessment Office, Research Triangle Park, NC, 1986.
19. EPA. Supplement to the 2086 EPA Air Quality Criteria for Lead, Vol. 1, Addendum. EPA/600/8-89/049A, U.S. Environmental Protection Agency, Office of Health and Environmental Assessment, Washington, DC, 1989.
20. Goyer, R. A. Lead toxicity: from overt to subclinical to subtle health effects. Environ. Health Perspect. 86: 177-181 (1990).
21. Landrigan, P. J. Lead in the modern workplace. Am. J. Public Health 80: 907-908 (1990).
22. Lippmann, M. Review: lead and human health: background and recent findings. Environ. Res. 51: 1-24 (1990).
23. Needleman, H. L. The persistent threat of lead: A singular opportunity. Am. J. Public Health 79: 643-645 (1989).
24. Needleman, H. L. The future challenge of lead toxicity. Environ. Health Perspect. 89: 85-90 (1990).
25. De la Burde, B., and Choate, M. S. Does asymptomatic lead exposure in children have latent sequelae? J. Pediatr. 81: 1088-1096 (1972).
26. De la Burde, B., and Choate, M. S. Early asymptomatic lead exposure and development at school age. J. Pediatr. 87: 638-664 (1975).
27. Rummo, J. H., Routh, D. K., Rummo, N. J., and Brown, J. F. Behavorial and neurological effects of symptomatic and asymptomatic lead exposure in children. Arch. Environ. Health 34: 120-124 (1979).
28. Grant, L. D., and Davis, J. M. Effects of low-level lead exposure on paediatric neurobehavioral development: current findings and future directions. In: Lead Exposure and Child Development (M. A. Smith, L. D. Grant, and A. I. Sors, Eds.), Kluwer Academic Publishers, Boston, MA, 1989, pp. 49-118.
29. Needleman, H. L., and Gatsonis, C. A. Low-level lead exposure and the IQ of children. A meta-analysis of modern studies. J. Am. Med. Assoc. 263: 673-678 (1990).
30. Winneke, G., Brockhaus, A., Ewers, U., Kramer, U., and Neuf, M. Results from the European Multicenter study on lead neurotoxicity in children: implications for risk assessment. Neurotoxicol. Teratol. 12: 553-559 (1990).
31. Bellinger, D., Leviton, A., Waternaux, C., Needleman, H., and Rabinowitz, M. Longitudinal analyses of prenatal and postnatal lead exposure and early cognitive development. N. Engl. J. Med. 316: 1037-1043 (1987).
32. Bellinger, D., Sloman, J., Leviton, A., Rabinowitz, M., Needleman, H. L., and Waternaux, C. Low-level lead exposure and children's cognitive function in the preschool years. Pediatrics 87: 219-227 (1991).
33. Deitrich, K. N., Succop, P. A., Berger, O. G., Hammond P. B., and Bornschein, R. L. Lead exposure and the cognitive development of urban, preschool children: the Cincinnati lead study cohort at 4 years. Neurotoxicol. Teratol. 13: 203-211 (1991).
34. McMichael, A. J., Baghurst, P. A., Wigg, N. R., Vimpani, G. V., Robertson, E. F., and Roberts, R. J. Port Pirie Cohort study: environmental exposure to lead and children's abilities at the age of four years. N. Engl. J. Med. 391: 468-475 (1988).
35. Ernhart, C. B., Morrow-Tlucak, M., Wolf, A. W., Super, D., and Drotar, D. Low level lead exposure in the prenatal and early preschool periods: intelligence prior to school entry. Neurotoxicol. Teratol. 11: 161-170 (1989).
36. Rothenberg, S. J., Schnaas, L., and Mendes, C. J. N. Effects of lead on neurobehavioral development in the first thirty days of life. In: Lead Exposure and Child Development (M. A. Smith, L. D. Grant, and A. I. Sors, Eds.), Kluwer Academic Publishers, Boston, MA, 1989, pp. 387-397.
37. Bellinger, D., Leviton, A., and Sloman, J. Antecedents and correlates of improved cognitive performance in children exposed *in utero* to low levels of lead. Environ. Health Perspect. 89: 5-12 (1990).
38. Needleman, H. L., Schell, A., Bellinger, D., Leviton, A., and Allred, E. N. Long term effects of childhood exposure to lead at low dose: an eleven-year follow-up report. N. Engl. J. Med. 322: 82-88 (1990).
39. Smith, M., Delves, T., Lansdowne, R., Clayton, B., and Graham, P. The effects of lead exposure on urban children. Dev. Med. Child Neurol. (suppl.) 47: 1-54 (1983).

40. Goldstein, G. W. Lead poisoning and brain cell function. Environ. Health Perspect. 89: 91–94 (1990).
41. Markovac, J., and Goldstein, G. W. Lead activates protein kinase C in immature rat brain microvessels. Toxicol. Appl. Pharmacol. 96: 14–23 (1988).
42. Rossouw, J., Offermeier, J., and von Rooyen, J. M. Apparent central neurotransmitter receptor changes induced by low-level lead exposure during different development phases in the rat. Toxicol. Appl. Pharmacol. 91: 132–139 (1987).
43. Holtzman, D., DeVries, C., Nguyan, H., Olson, J., and Bensch, K. Maturation of resistance to lead encephalopathy: cellular and subcellular mechanisms. Neurotoxicology 5: 97–124 (1984).
44. Goyer, R. A. Lead toxicity: a problem in environmental pathology. Am. J. Pathol. 64: 167–182 (1971).
45. Moore, J. F., Goyer, R. A., and Wilson, M. H. Lead-induced inclusion bodies: solubility amino acid content and relationship to residual acidic nuclear protein. Lab. Invest. 29: 488–494 (1973).
46. Egle, P., and Shelton, K. R. Chronic lead intoxication causes a brain-specific nuclear protein to accumulate in the nuclei of cells lining kidney tubules. J. Biol. Chem. 261: 2294–2298 (1986).
47. Harlan, W. R., Landis, J. R., Schmouder, R. L., Goldstein, N. G., and Harlan, L. C. Blood lead and blood pressure: relationship in the adolescent and adult population. J. Am. Med. Assoc. 253: 530–534 (1985).
48. Pirkle, J. L., Schwartz, J., Landis, J. R., and Harlan, W. R. The relationship between blood lead levels and blood pressure and its cardiovascular risk implications. Am. J. Epidemiol. 121: 246–258 (1985).
49. Sharp, D. S., Osterloh, J., Becker, C. E., Bernard, B., Smith, A. H., Fisher, J. M., Syme, S. L., Holamn, B. L., and Johnston, T. Blood pressure and blood lead concentration in bus drivers. Environ. Health Perspect. 78: 131–137 (1989).
50. Schwartz, J. Lead, blood pressure, and cardiovascular disease in men and women. Environ. Health Perspect. 91: 71–76 (1991).
51. Chai, S., and Webb, R. C. Effects of lead on vascular reactivity. Environ. Health Perspect. 78: 85–89 (1988).
52. Vander, A. J. Chronic effects of lead on the renin-angiotensin system. Environ. Health Perspect. 78: 77–83 (1988).
53. Silbergeld, E. K. Lead in bone: implications for toxicology during pregnancy and lactation. Environ. Health Perspect. 91: 63–70 (1991).
54. Rabinowitz, M. D. Toxicokinetics of bone lead. Environ. Health Perspect. 91: 33–38 (1991).
55. Pounds, J. G., Long, G. J., and Rosen, J. F. Cellular and molecular toxicity of lead in bone. Environ. Health Perspect. 91: 17–32 (1991).
56. Thomas, B. J. Equipment design issues for the *in vivo* X-ray fluorescence analysis of bone lead. Environ. Health Perspect. 91: 39–44 (1991).
57. Rosen, J. F., Markowitz, M. E., Bijur, P. E., Jenks, S. T., Wielopolski, L., Kalef-Ezra, J. A., and Slatkin, D. N. Sequential measurements of bone lead content by X-ray fluorescence in $CaNa_2$ EDTA-treated lead-toxic children. Environ. Health Perspect. 91: 57–62 (1991).
58. Telisman, S., Cvitkovic, P., Gavella, M., and Pongracic, J. Semen quality in men with respect to blood lead and cadmium levels. In: International Symposium on Lead and Cadmium Toxicology. Peking, People's Republic of China, August 18–21, 1990, pp. 29–32.
59. Foster, W. G. Reproductive toxicity of chronic lead-exposure in the female cynomolgus monkey. Reprod. Toxicol., 6: 123–131 (1992).
60. Baker, E. L., Goyer, R. A., Fowler, B. A., Khattr, U., Bernard, O. B., Adler, S., White, R., Babayan, R., and Feldman, R. G. Occupational lead exposure, nephropathy and renal cancer. Am. J. Med. 1: 139–148 (1980).
61. Lilis, R. Long-term occupational lead exposure: chronic nephropathy and renal cancer: a case report. Am. J. Ind. Med. 2: 293–297 (1981).
62. EPA. Evaluation of the Potential Carcinogenicity of Lead and Lead Compounds. EPA/600/8-89/045A, U.S. Environmental Protection Agency, Office of Health and Environmental Assessment, Washington, DC, 1989.
63. Goyer, R. A. Nephrotoxicity and carcinogenicity of lead (summary of symposium presentation). Fundam. Appl. Toxicol. 18: 4–7 (1992).
64. Selevan, S. G., Landrigan, P. J., Stern, F. B., and Jones, J. H. Mortality of lead smelter workers. Am. J. Epidemiol. 122: 673–683 (1985).
65. Cooper, W. C., Wong, O., and Kheifets, L. Mortality among employees of lead battery plants and lead-producing plants, 1947–1980. Scand. J. Work Environ. Health 11: 331–345 (1985).
66. Goyer, R. A. The nephrotoxic effects of lead. In: Nephrotoxicity (P. H. Bach, F. W. Bonner, J. W. Bridges, and E. A. Lock, Eds.), John Wiley and Sons, Chichester, England, 1982, pp. 338–348.
67. Buchet, J. P., Roels, H., Bernard, A., and Lauwerys, R. Assessment of renal function of workers exposed to inorganic lead, cadmium or mercury vapor. J. Occup. Med. 22: 741–752 (1980).
68. Cramer, K., Goyer, R. A., Jagenburg, O. R., and Wilson, M. H. Renal ultrastructure, renal function and parameters of lead toxicity in workers with different length of lead exposure. Br. J. Ind. Med. 31: 113–127 (1974).
69. Moore, M. R., and Meredith, P. A. The carcinogenicity of lead. Arch. Toxicol. 42: 87–94 (1979).
70. Mao, P., and Molnar, J. J. The fine structure and histochemistry of lead-induced renal tumors in rats. Am. J. Pathol. 50: 571–603 (1967).
71. Van Esch, G. J., and Kroes, R. The induction of renal tumors by feeding basic lead acetate to mice and hamsters. Br. J. Cancer 23: 765–771 (1969).
72. Azar, A., Trochimowicz, H. J., and Maxfield, M. E. Review of lead studies in animals carried out at Haskell Laboratory: two-year feeding study and response to hemorrhage study. In: Environmental Health Aspects of Lead. Proceedings of an International Symposium, Amsterdam, Luxembourg. Commission of the European Communities, Luxembourg, 1973, pp. 199–210.
73. NCI. Bioassay of Lead Dimethyldithiocarbamate for Possible Carcinogenicity. Technical Report Series No. 151, National Cancer Institute, U.S. Department of Health and Human Services, Washington, DC, Pb-85-134663, 1979.
74. Goyer, R. A., Leonard, D. L., Moore, J. F., Rhyne, B., and Krigman, M. R. Lead dosage and the role of the intranuclear inclusion body. Arch. Environ. Health 20: 705–711 (1970).
75. Cantor, K. P., Sontag, J. M., and Held, M. F. Patterns of mortality among plumbers and pipefitters. Am. J. Ind. Med. 10: 73–89 (1986).
76. IARC. Monographs on the Evaluation of Carcinogenic Risks to Humans: An updating of IARC Monographs 1 to 42, Supplement 7. International Agency for Research on Cancer, Lyon, France, 1987, pp. 230–231.
77. Zelikoff, J. T., Li, J. H., Hartwig, A., Wang, X. W., Costa, M., and Rossman, T. G. Genetic toxicology of lead compounds. Carcinogenesis 9: 1727–1732 (1988).
78. Stoner, G. D., Shimkin, M. B., Troxell, M. C., Thompson, T. L., and Terry, L. S. Test for carcinogenicity of metallic compounds by the pulmonary tumor response in strain A mice. Cancer Res. 36: 1744–1747 (1976).
79. Oyasu, R., Battifora, H. A., Clasen, R. A., McDonald, J. A., and Hass, G. M. Induction of cerebral gliomas in rats with dietary lead subacetate and 2-acetylaminofluorene. Cancer Res. 30: 1248–1261 (1970).
80. Hinton, D. E., Lipsky, M. M., Heatfield, B. M., and Trump, B. F. Opposite effects of lead on chemical carcinogenesis in kidney and liver of rats. Bull. Environ. Contam. Toxicol. 23: 46–469 (1979).
81. Choie, D. D., and Richter, G. W. Cell proliferation in rat kidney induced by lead acetate and effects of uninephrectomy on cell proliferation. Am. J. Pathol. 66: 265–275 (1972).
82. Choie, D. D., and Richter, G. W. Cell proliferation in mouse kidney induced by lead. I. Synthesis of deoxyribonucleic acid. Lab. Invest. 30: 647–651 (1974).
83. Columbano, A., Ledda-Columbano, G. M., Lee, G., Rajalakshmi, S., and Sarma, D. S. R. Inability of mitogen-induced liver hyperplasia to support the induction of enzyme-altered islands induced by liver carcinogens. Cancer Res. 47: 5557–5559 (1984).
84. Bernstein, J., Evan, A. P., and Gardner, K. D. Epithelial hyperplasia in human polycystic kidney disease. Its role in pathogenesis and risk of neoplasia. Am. J. Pathol. 129: 92–101 (1987).
85. Goyer, R. A., Falk, H. L., Hogan, M., Feldman, D. D., and Richter, W. Renal tumors in rats given trisodium nitrilotriacetic acid in drinking water for 2 years. J. Natl. Cancer Inst. 66: 869–880 (1981).
86. Dees, J. H., Heatfield, B. M., Reuben, M. D., and Trump, B. F. Adenocarcinoma of the kidney, III. Histogenesis of renal adenocarcinomas induced in rats by N-(4-fluoro-4-biphenyl)acetamide. J. Natl. Cancer Inst. 64: 1537–1545 (1980).
87. Roberts, P. F. Bilateral renal carcinoma associated with polycystic kidneys. Br. Med. J. 3: 273–274 (1973).
88. Turani, H., Levi, J., Zevin, D., and Kessler, E. Acquired cystic disease and tumors in kidneys of hemodialysis patients. Isr. J. Med. Sci. 19: 614–618 (1983).
89. Barry, P. S. I. Concentrations of lead in the tissues of children. Br. J. Ind. Med. 38: 61–71 (1981).
90. Barry, P. S. I. A comparison of concentrations of lead in human tissues. Br. J. Ind. Med. 32: 119–139 (1975).
91. Mahaffey, K. R. Environmental lead toxicity: nutrition as a component of intervention. Environ. Health Perspect. 89: 75–78 (1990).
92. Barltrop, D. Transfer of lead in the human foetus. In: Mineral Metabolism in Pediatrics (D. Barltrop and W. Burland, Eds.), Blackwell Scientific, Oxford, 1969, pp. 135–151.
93. Bonithon-Kopp, C., Huel, G., Grasmick, C., Sarmini, H., and Moreau, T. Effects of pregnancy on the inter-individual variation in blood levels of lead,

cadmium and mercury. Biol. Res. Preg. 7: 37–42 (1986).
94. Oski, F. A., Honig, A. S., Hely, B., and Howanitz, P. Effects of iron therapy on behavior performance in non-anemic iron deficient infants. Pediatrics 71: 877–880 (1983).
95. Cerklewski, F. L., and Forbes, R. M. Influence of dietary zinc on lead toxicity in the rat. J. Nutr. 106: 689–696 (1976).
96. ASTDR. The Nature and Extent of Lead Poisoning in Children in the United States: A Report to Congress. Agency for Toxic Substances and Disease Registry, U.S. Department of Health and Human Services, Atlanta, GA, 1988.
97. DHHS. Healthy People 2000, the Year 2000: National Health Promotion and Disease Prevention Objectives. U.S. Department of Health and Human Services, Washington, DC, 1989.